Siliceous structures secreted by members of the subclass Lobosia (Rhizopodea: Protozoa)

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Introduction

The need for a comprehensive taxonomic system for those amoebae with shells, usually referred to as testate amoebae, is long overdue. Nevertheless, before such a task is undertaken the valid criteria for each taxon should be established. The two main criteria used in recent classifications of testate amoebae in the Superclass Rhizopodea are the cytoplasmic form of the pseudopodia and the structure of the shell. The present study is concerned only with the latter, so the proposals concerning the mechanisms of pseudopodial movement suggested by Bovee & Jahn (1966) have not been considered. Furthermore, the changes put forward here are modifications of the classifications of Loeblich & Tappan (1961, 1964) and Deflandre (1953) – (referred to as the 'legally-based system' by Bovee & Jahn). In these classifications the shape and composition of the shell has been used to determine the divisions at the superfamily and family level. Four basic types of shell are recognized : first, the proteinaceous shell, usually composed of numerous alveoli; secondly, the agglutinate shell composed of extraneous material bound by an organic cement; thirdly, the siliceous shell usually composed of shell plates; and finally, the calcareous shell which may have an outer organic lining.

The divisions proposed here relate to the third type of shell structure, and concern those animals which make their own siliceous shell components. To date, that includes most of the species belonging to the subclass Filosia, and some species belonging to the subclass Lobosia, namely *Difflugia oviformis* Cash, 1909, *Quadrulella symmetrica* (Wallich, 1863) and *Lesquereusia spiralis* (Ehrenberg, 1840). Other testate amoebae in the subclass Lobosia which have siliceous plates, for example, species belonging to the genera *Nebela* and *Heleopera* have agglutinate shells, made of captured shell plates or quartz particles. The shell plates of smaller testate amoebae, such as *Euglypha*, *Assulina* and *Cyphoderia*, are easily recognized in the shells of *Nebela* species, and MacKinlay (1936) suggested that these were obtained by predation after observing that *Nebela collaris* produced a membranous shell devoid of shell plates when grown in isolation.

Discussion

It is generally accepted that all testate amoebae secrete an organic material that either provides the entire shell, as in *Arcella*, or at least is used to cement the shell components together as in, for example, *Nebela*, *Difflugia* and *Euglypha*. Fine structure studies (Hedley & Ogden, 1973, 1974*a*; Netzel, 1975, 1976*a*, 1977; Hedley *et al*., 1976, 1977) on species belonging to all four of the basic shell types have shown that this organic material is made in the Golgi complex of the cytoplasm, and have suggested how it is probably used in shell construction. In addition, the cytoplasmic changes associated with the formation and distribution of siliceous structures in *Euglypha rotunda*, *E. acanthophora*, *E. strigosa*, *Trinema lineare* and *Difflugia oviformis* have been described in detail by Hedley & Ogden (1973, 1974*a*, *b*) and Netzel (1976*b*, 1977). These studies, based on laboratory cultures, include observations on the biology of these species, and cine-films illustrating binary fission have been made by Netzel (1971*a*, *b*). The siliceous structures secreted by *Euglypha* species and *Difflugia oviformis* are formed in a membrane-bound vesicle within the cytoplasm, and are stored there prior to division. At division, they are arranged around a cytoplasmic extrusion to form a daughter shell identical to the parent. The term 'idiosomes' has been suggested (Netzel, 1972) for the siliceous structures secreted by *Difflugia oviformis*.

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Using the diagnosis of the family Difflugiidae given by Loeblich & Tappan (1961, 1964), that the 'test is rarely chitinous, but is generally composed of foreign particles, not of secreted plates' and of the genus *Difflugia* – 'wall with pseudochitinous base and variable amounts of agglutinate material', it is apparent that *Difflugia oviformis* with its secreted shell does not agree with this definition. There would appear to be no justification in amending the diagnosis for an already overcrowded genus, instead it is proposed to create a new genus *Netzelia* to include *D. oviformis* and other species of *Difflugia* which secrete their own siliceous elements or idiosomes.

A similar situation arises in the family Hyalospheniidae. The diagnosis for this family states – 'test chitinous with siliceous plates or scales, rounded or angular, and may have foreign matter added'. Two of the genera included in this family are thought to secrete a proteinaceous shell, *Hyalosphenia* Stein, 1857 and *Leptochlamys* West, 1901, and two siliceous structures, *Quadrullella* Cockerell, 1909 and *Lesquereusia* Schlumberger, 1845. An ultrastructural study of *Hyalosphenia papilio* by Joyon & Charret (1962) suggested that it had a proteinaceous shell, but little is known about the shell of *Leptochlamys* except that it was originally described as having a thin chitinous membrane. Information on the shell of *Quadrulella* is still needed, although it is assumed that it secretes its own shell plates, because they have a distinct quadrangular shape (Fig. 1) which is restricted to this genus and *Paraquadrula* Deflandre, 1932 which has calcareous plates. Species belonging to *Quadrulella* have previously been described as having calcareous, siliceous or chitinoid shells (Cash & Hopkinson, 1909 ; 134–135). Elemental analysis of whole shells of *Q. symmetrica*, recently carried out in this laboratory using an energy dispersive X-ray analyser attached to a scanning electron microscope, have shown that they are composed mainly of silicon, with some potassium and calcium present. The amounts detected suggest that the shell plates are siliceous and that the organic cement which binds them may be strengthened by the other elements. The genus *Lesquereusia* contains several species whose shells are composed mainly of siliceous rods, three of these *L. epistomum*, *L. modesta* and *L. spiralis* have recently been redescribed (Ogden & Hedley, 1980) and only *L. modesta* consistently incorporates irregular foreign particles amongst the rods in the shell.

Although clonal cultures of *Lesquereusia spiralis* have not yet been established, observations of this species in isolation, in this laboratory, have shown that it secretes a daughter shell made of curved rods similar to those of the parent. Some of the laboratory specimens were smaller than those reported from the wild (see Ogden & Hedley, 1979), and this is thought to be due to a deficiency in the culture medium. A comparison between the curved rods of the smallest specimen, 83 µm (Fig. 2) those of a medium specimen, 95 µm (Figs 4 & 5) and a large specimen, 120 µm (Figs 6 & 7), show that these rods become more pronounced and areas of organic cement with the distinctive pores (Fig. 3) less numerous with increase in size. Elemental analysis of whole shells, using an energy dispersive X-ray analyser, has shown that these rods are siliceous and suggests that the organic cement that binds them contains iron.

The situation regarding the family Hyalospheniidae needs some clarification, but must await studies on clonal cultures before this is possible. However, it seems to me that there are sufficient affinities between *Lesquereusia spiralis*, *Quadrulella symmetrica* and the new genus *Netzelia* to

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Fig. 1 Siliceous shell plates of *Quadrulella symmetrica*. × 2400

Fig. 2 Shell surface of small specimen of *Lesquereusia spiralis*, to show the curved rods and pores. × 2100

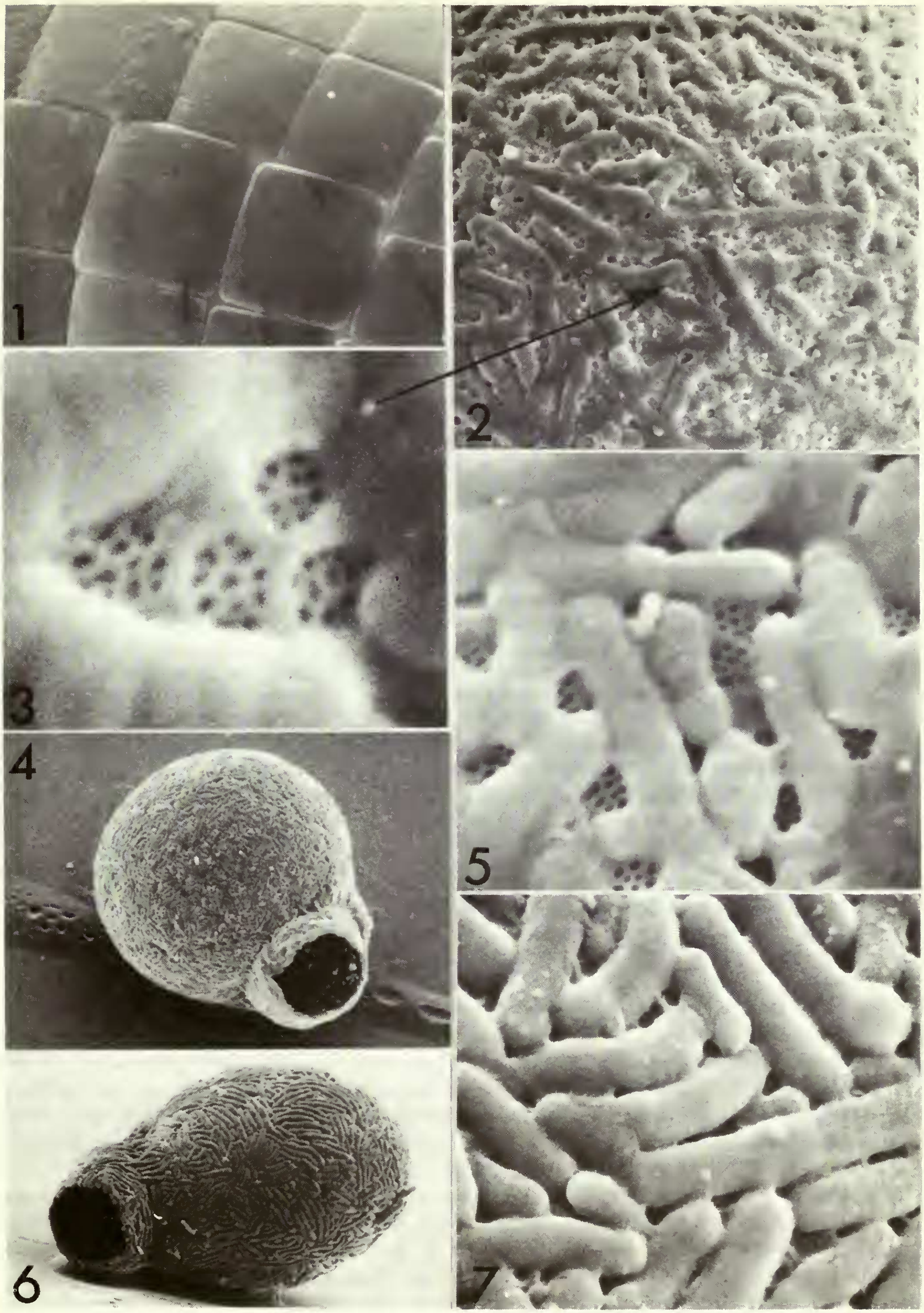
Fig. 3 A higher magnification of the pores in the organic cement, from the area arrowed in Fig. 2. × 21 000

Fig. 4 A medium-sized specimen of *L. spiralis* to show the ill-defined siliceous rods. × 490

Fig. 5 Portion of shell surface of medium specimen, note the numerous pores. × 8900

Fig. 6 Large specimen of *L. spiralis* with well-defined siliceous rods. × 480

Fig. 7 Shell surface of large specimen, note the apparent absence of pores. × 4800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warrant family status within the subclass Lobosia. I therefore propose to designate a family to accommodate these species by redefining Lesquereusiidae Jung, 1942, previously synonymized by Loeblich & Tappan (1961), to include those members of the subclass Lobosia which secrete siliceous elements.

Family **LESQUEREUSIIDAE** Jung, 1942 nom. rev.
[nom. correct pro Lecuereusiidae Jung, 1942]

Shell constructed of siliceous rods, plates or idiosomes secreted in the cytoplasm, to which mineral particles may be added; aperture circular, oval or elongate.

Lesquereusia Schlumberger, 1845. Shell colourless, compressed ovoid or globose with asymmetrical neck, giving the appearance of a spiral, composed of interwoven siliceous rods and sometimes particles of quartz; aperture circular.

to include: *Lesquereusia epistomum* Penard, 1902
Lesquereusia modesta Rhumbler, 1895
Lesquereusia spiralis (Ehrenberg, 1840)

Netzelia gen. n. Shell colourless, ovoid, symmetrical, with a broad crown and sides tapering smoothly to the aperture, composed of idiosomes but may incorporate grains of quartz; aperture circular, with organic collar and often with four or five small lobes.

to include: *Netzelia oviformis* (Cash, 1909) comb. n.

Quadrulella Cockerell, 1909. Shell colourless, ovoid, composed of quadrangular shell plates arranged without overlapping; aperture oval.
to include: *Quadrulella symmetrica* (Wallich, 1863).

In defining the above taxonomic changes, none of the generic names proposed by Jung (1942) in his review of the genus *Difflugia* have been considered, I agree with Deflandre (1953) and subsequent authors that these must be rejected as inadequate definitions.

It is apparent from previous descriptions of species of *Difflugia* that problems have arisen in identification due to differences of shell structure. For example, Penard (1902) described specimens similar to *Difflugia tuberculata* (Wallich, 1864) but having a thin, transparent shell without indentations. These specimens are listed by Jung (1942) as *Cingodifflugia laevis* (Penard, 1902). A species of *Difflugia* collected from water and mud in Alabama was tentatively identified by Owen & Jones (1976) as *D. tuberculata*. It produced autogenous siliceous components when isolated in culture, and Owen & Jones suggested that this species should therefore be referred to the genus *Nebela*. Their specimens do not agree with the accepted definition of *D. tuberculata*, which typically have distinctive tubercles of quartz grains on the shell surface (see Ogden & Hedley, 1979). However, this latter description may have to be amended because examination of specimens from the Everglades National Park, Florida, U.S.A. (kindly collected by Dr C. R. Curds, British Museum (Natural History)), suggest that *D. tuberculata* may occasionally construct a shell of diatom frustules instead of the usual quartz particles, both examples being present in the sample. Nevertheless, I consider that the descriptions of Penard (1902), Jung (1942) and Owen & Jones (1976) all refer to specimens of *Netzelia*.

It is probable that other smooth-shell species of *Difflugia* may secrete siliceous elements, although observations on such species in culture are needed. For example, the study of the shell structure of *Difflugia lobostoma* given by Eckert & McGee-Russell (1974) must be considered with caution, as I believe from their description that these authors were studying specimens of *D. tuberculata*. Nevertheless, the specimens examined were composed of a single layer of siliceous particles held together by a network of cement, and dense granules similar in structure to those seen in *Netzelia oviformis* by Netzel (1976*b*) were observed between the shell joints and in the cytoplasm.

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